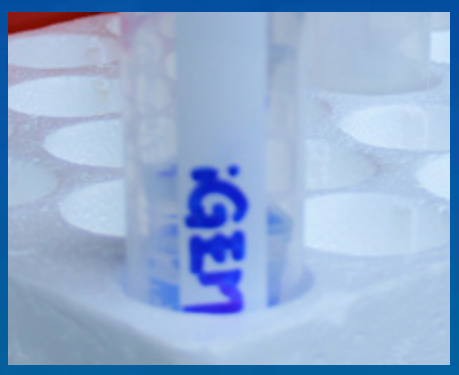
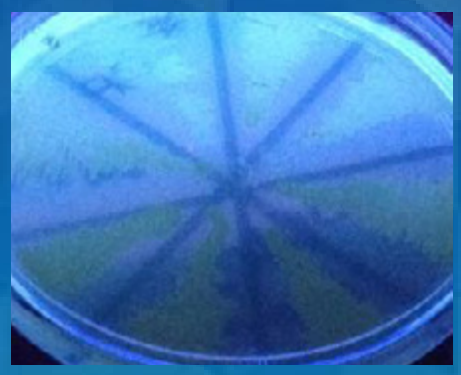
GOOD LABORATORY PRACTICE

AN iGEM LABORATORY WORK HANDBOOK



VIRTUS PARVA

TEC-CEM

Foreword

Working with the handbook and manuals became and inspiring piece of work. Because the work put into them was, primarily, in order to help and assess new teams and experienced ones as well. Several parts became involved in the creation of these materials, as the original holders of the idea, the ITESM campus CEM team knowing this would not be an easy walk, sought co-authorship. That's when the Virtus Parva team decided to become involved and help each other out. There are three handbooks, each, with a specific area: the Good Laboratory Practice handbook, which helps on setting the standards of the work done in the laboratory; the iGEM Registry handbook, which is an easy to follow step guide for all those who venture into the iGEm parts domain, it covers from looking up a standard part into adding your own, going through the sending of a part for sequencing and the shipping process; and last but not least, the iGEM team handbook, this one hopes to cover most of the team formation process, as well to be a guideline for teams to follow along the process until the Jamboree is concluded. This work was the most complex to resolve, still, some teams may not find it insightful since there are many ways a team responds and stays motivated.

Good Laboratory Practice (GLP) embodies a set of principles that provides a framework within which laboratory studies are planned, performed, monitored, recorded, reported and archived. These studies are undertaken to generate data by which the hazards and risks to users, consumers and third parties, including the environment, can be assessed for pharmaceuticals (only preclinical studies), agrochemicals, cosmetics, food additives, feed additives and contaminants, novel foods, biocides, detergents etc.... GLP helps assure regulatory authorities that the data submitted are a true reflection of the results obtained during the study and can therefore be relied upon when making risk/safety assessments.

As set by the Medicines and Healthcare Regulations Agency (MHRA), that is the actual definition of GLP. this handbook will aid students and instructors alike in the application of such standarts, though it is not as thorough as the WHO GLP Handbook, it is a neat approach into the environment and work policies that must be followed in order to assure a quality procedure.

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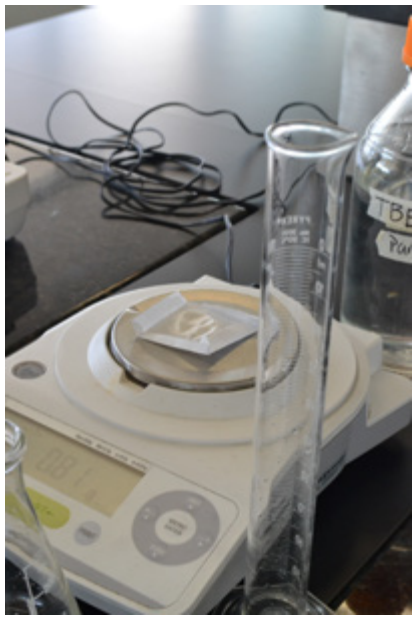
Subpart A: General Provisions



This part prescribes good laboratory practices for conducting through iGEM competition. They are intended to support applications for researching in biology synthetic in topics of: energy, environment, food and nutrition, manufacturing, health and medicine, and software into services or products regulated by the Food and Drug Administration. Compliance with this part is intended to assure the quality and integrity of the safety data filed. Another general issues to consider are:

a) When work is in progress, laboratory doors must be closed.

b) All the iGEM team members must wear personal protective equipment such as: laboratory coats or gowns, disposable gloves where required, and always protective goggles at the remotest risk for the eyes.

c) Eating, drinking, smoking, snuffing, applying of cosmetics and storing of food, drinks and tobacco are absolutely prohibited in the laboratory

d) Mouth pipetting is prohibited! Mechanical pipetting instruments should be used instead.

e) Minimize the generation of aerosols.

f) Use syringes and cannulas only if absolutely necessary.

g) Personnel wash their hands before and after finishing work.

h) Keep laboratory tidy and clean; only instruments and material actually required in the work process are to be placed on workbenches; keep stocks of regulated material in separate storage rooms or cabinets, if possible.

Subpart B: Organization and Personnel

A. About the Personnel and group members

a) Each individual engaged in the conduct of or responsible for the supervision of the iGEM laboratory study shall have education, training, and experience, or combination thereof into the biotechnology, molecular biology, genetic engineering, or life science areas; to enable that individual to perform the assigned functions.

b) Each testing facility shall maintain a current summary of training and experience and job description for each individual engaged in or supervising the conduct of the iGEM laboratory study.

c) There shall be a sufficient number of personnel for
the timely and proper conduct of the study according to the protocol.

d) Personnel shall take necessary personal sanitation and health precautions designed to avoid contamination of test and control articles and test systems.

e) Personnel engaged in the study shall wear clothing appropriate for the duties they perform. Such clothing shall be changed as often as necessary to prevent microbiological, radiological, or chemical contamination of test systems and test and control articles. In the present case, the use of lab coat, closed shoes, clean hands and hair up are a daily work requirement

f) Any team member founded at any time to have an illness that may adversely affect the quality and integrity of the iGEM laboratory study shall be excluded from direct contact with test systems, test and control articles and any other operation or function that may adversely affect the study until the condition is corrected. Those person is responsible for notify to the team leader the cause of the affliction and report their absence.

B. Testing facility management.

a) Assure that personnel, resources, facilities, equipment, materials, and methodologies are available as scheduled.

b) Assure that each team member clearly understand the functions they are to perform.

C. Responsibilities of the Instructors and team members

a) For each iGEM laboratory study, a scientist or other professional of appropriate education, training, and experience, or combination thereof, shall be identified as an Instructors of the iGEM team. An Instructor has overall responsibility for the technical conduct of the interlab and be completely accessible in case of doubts at some protocols. Also, all the team members are responsible of the interpretation, analysis, documentation and reporting of results. The leaders of the interlab, wiki and biosafety must assure that:

i. All experimental data, including observations of unanticipated responses of the test system are accurately recorded and verified.

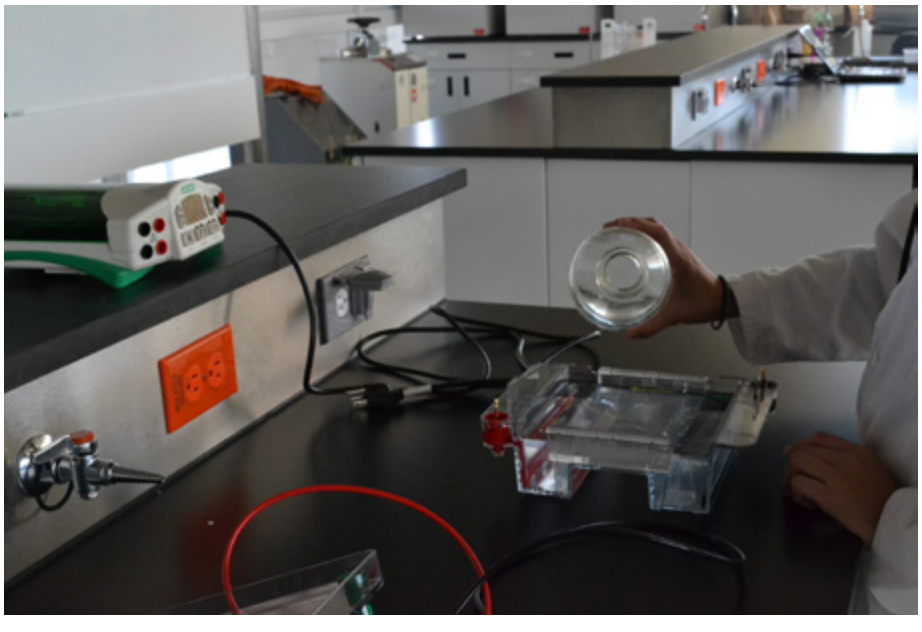
ii. Unforeseen circumstances that may affect the quality and integrity of the nonclinical laboratory study are noted when they occur, and corrective action is taken and documented.

iii. Test systems are as specified in the protocol.

iv. All applicable good laboratory practice regulations are followed.

v. All raw data, documentation, protocols, annotations, and final reports are transferred to the archives during or at the close of the presentation of the iGEM competition.

Subpart C: Facilities



A. General.

Each laboratory facility shall be of suitable size and construction to facilitate the proper conduct of iGEM laboratory studies. It shall be designed so that there is a degree of separation that will prevent any function or activity from having an adverse effect on the study.

B. Facilities for handling test and control articles.

a) As necessary to prevent contamination or mixups, there shall be separate areas for:

i. Receipt and storage of the tests and control articles.
ii. Mixing of the test and control articles
iii. Storage of the test and control article mixtures.

b) Storage areas for the test and/or control article and test and control mixtures shall be separate from areas housing the test systems and shall be adequate to preserve the identity, strength, purity, and stability of the articles and mixtures such as:

i. Reagent preparation area.
This includes DNA/RNA extracts, samples, cloned materials and PCR products).
ii. The nucleic acid extraction area
iii. The amplifications area.
The amplification room is the area in which the PCR machines are housed. Cloned DNAs should not be brought into this area.Where PCR machines are shared, a clear booking system is recommended to provide a cohesive system for the assays. Individual users' PCR programs in the thermocyclers should not be edited by other users (even temporarily) without notification to the original program team owner.
iv. The product analysis rooms.
This is the room in which post-PCR manipulations are performed eg agarose gel electrophoresis of products, PCR-ELISA detection systems. This is a contaminated area and therefore no reagents, equipment, laboratory coats etc. from this room should be used in any of the other PCR areas.

C. Laboratory operation areas.

Separate laboratory space shall be provided, as needed, for the performance of the routine and specialized procedures required by molecular and synthetic biology studies.

D. Specimen and data storage facilities.
Space shall be provided for archives, notes and drafting of documents in every moment, limited to access by authorized personnel (for the leaders of the interlab, wiki and biosafety only) , in order to establish a storage and retrieval of all raw data and specimens from completed studies.

Subpart D: Equipment

A. Equipment design.

Equipment used in the generation, measurement, or assessment of data and equipment used for facility genetic material control shall be of appropriate design and adequate capacity to function according to the protocol and shall be suitably located for operation, inspection, cleaning, and maintenance such the: thermocycler, microwave, power supplies, vortex, micropipettes, etc.

B. Maintenance and calibration of equipment.

a) Equipment shall be adequately inspected, cleaned, and maintained. Equipment used for the generation, measurement, or assessment of data shall be adequately tested, calibrated and/or standardized.

b) Written records shall be maintained of all inspection, maintenance, testing, calibrating and/or standardizing operations. These records, containing the date of the operation, shall describe whether the maintenance operations were routine and followed the written standard operating procedures. Written records shall be kept of non routine repairs performed on equipment as a result of failure and malfunction. Such records shall document the nature of the defect, how and when the defect was discovered, and any remedial action taken in response to the defect.

Subpart E: Testing Facilities Operation

A. Standard operating procedures

The different areas in the lab must have different standard procedures to insure the quality and integrity of the results generated from the project.

i. When working in the laminar flow hood be sure to sanitize your hands and any materials entering the hood, be sure that any external currents can't enter your area of work, and that you are working with sterile equipment (glassware, micropipette tips, etc.).

ii. When working with enzymes be sure to keep them at room temperature for the absolutely necessary length of time and return them immediately to freezing temperature.

iii. When working with hot equipment such as boiling water be sure to wear the protective gear necessary and after its use leave it close to the area that requires it.

iv. Have a unidirectional workflow that insures your sterile areas won't be contaminates, always work from sterile zones to not sterile zones and never the other way around, also try to change your lab gear when changing zones if possible.

b. Any deviation from the standard procedures should be authorized by team instructors.

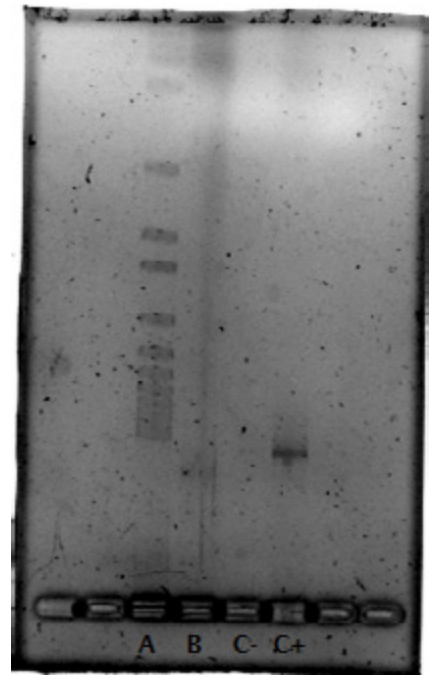
c. Each laboratory area shall have immediately available laboratory handbooks and standard operating procedures relative to the laboratory procedures being performed.

B. Reagents and solutions

Reagents should be prepared and stored in a biological agents free zone to prevent its contamination, such biological agents could be but are not limited to:

i. DNA/RNA extracts.
ii. Samples.
iii. Cloned materials.
iv. PCR products.

Aliquotation of master mixes and stocks should be done in case a contamination occurs.



Subpart F: Records and Reports

A) A final report of the results obtained should include:

a. Name and working address of the iGEM team performing the experimentation and the dates on which it began and ended.

b. Objectives and final procedures used, including any changes from the original protocols.

c. Any mathematical method used for analyzing the data.

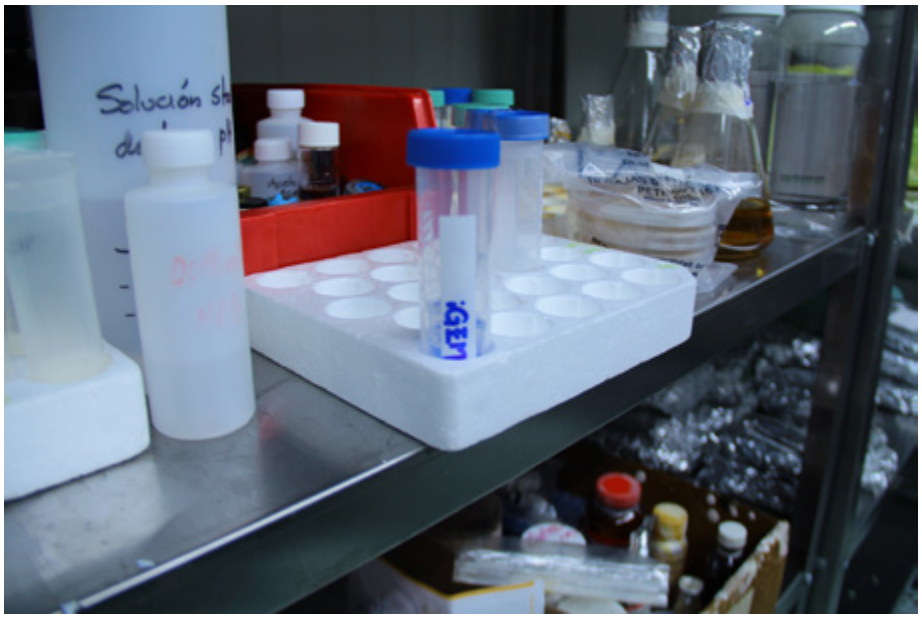
d. A description of the test system used.

e. A description of all circumstances that may have affected the final results.

f. The names of all the iGEM team members, including instructors, that were involved in the project.

g. Final date and signs of all the iGEM team members, including instructors, that were involved in the project.

B) A daily report of all the activities done in the lab should be made for the team wiki, it should include all the major procedures realized and any problems that occurred in the lab or outside of it.



C) Any reactive, cell culture, bioparts, etc. should be correctly labeled

a. Labels should at least include:
   i. Date.
   ii. What it is.
   iii. An iGEM legend.

b. In some cases they could also include:
   i. Who did it.
   ii. Exact hour.
   iii. Its intended use.

D) Deteriorated or outdated reagents shouldn't be used.

E) A blog should be kept with all the protocols, procedures and activities done day to day, it should also contain specifics of any methodology or procedure done, such as volumes used to make a PCR or order in which substances were charged in a gel electrophoresis.

F) The schedule of work of team members, copies of protocols and all blogs should be accessible at any time in the lab for anyone authorized to consult.

REFERENCES

- http://www.gene-quantification.de/good-laboratory-practise-qpcr-qsop38-2010.pdf
- http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=58